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Opinion Article

# Assessment of the *in Vitro* Fertilization Process in Living Egg Cells using Artificial Insemination in Rodents

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## ABOUT THE STUDY

Previous research has used whole-mount preparation to observe the fertilization process in rats at various time points after insemination. However, very few reports have used an inverted microscope without a whole-mount to describe the various events during the fertilization process. Furthermore, no reports to our knowledge have described changes in sperm motility associated with sperm penetration into oocytes. In this study, frozen-thawed sperm from various rat strains (SD, Wistar, LE, F344, and BN) and oocytes from the SD strain were used for *in vitro* fertilization and the process of sperm penetration into the oocytes and subsequent development were observed. Sperm motility was evaluated, as well as the relationship between the process of sperm penetration into oocytes and sperm motility over time was investigated. The motility of frozen sperm from SD, Wistar, LE, and F344 increased 2-3 hours after thawing, when the sperm attached to the zona pellucida. Sperm penetrated the zona pellucida after 3-5 hours, and pronuclear formed in the cytoplasm of oocytes 5-9 hours after insemination. The fertilities of SD, Wistar, LE, and F344 frozen-thawed sperm were 92.7%, 90.0%, 90.7%, and 68.7%, respectively. However, there was no increase in motility after thawing frozen sperm from the BN, and fertility was only 21%. Furthermore, very few polyspermic oocytes were observed when frozen-thawed sperm from all strains was used. In conclusion, rats are suitable animals for observing sperm penetration into oocytes, and we used frozen-thawed rat sperm to determine the timing of fertilizations events in IVF. There have been numerous literature indications of IVF in rats since the first successful IVF experiment in rats using epididymal sperm. After whole-mount preparation, inseminated oocytes were observed under a phase-contrast microscope for fertilization assessment in all of these reports. Only one study has so far noted on observing the fertilizations process in living oocytes *in vitro* using fresh rat sperm. The fertilizations process of the oocytes, from sperm penetration to the formation of pronuclear, was simply observed through photography in that paper and was not studied in depth. Rat sperm is 3-4 times longer (total length, approximately 200 m) than that of domestic animals and humans. This makes it an appropriate animal species for studying the mammalian fertilizations process. However, due to the large number of zone-attached sperm and supplemented sperm (sperm that have entered the perivitelline space of oocytes) after insemination, and the occurrence of polyspermic fertilizations in many oocytes (0%-40%), it is difficult to observe living oocytes over time in the case of IVF using fresh sperm. We recently succeeded in cryopreserving rat sperm and successfully fertilizing eggs with frozen-thawed sperm in various strains of rats. Interestingly, we discovered that IVF with frozen-thawed rat sperm is a good model for studying the fertilizations process. This is due to the fact that few sperm are attached to the zona pellucida, and one sperm can penetrate through it and complete monospermic fertilizations. Sperm motility was measured using a computer-assisted sperm analyzer after cryopreservation and thawing Sperm were incubated in mHTF for up to 6 hours at 37°C in an atmosphere of 5% $CO_2$, and sperm motility was measured every hour with an IVOS analyzer. The following sperm motility parameters were evaluated: the proportion of motile sperm movement of more than 5 m/s and percentage of progressively motile sperm (motile sperm with a path velocity greater than 50 m/s and a straightness ratio greater than 50%). Path Velocity (VAP), Progressive Velocity (VSL), and track speed were also measured, as well as Lateral Amplitude of Head (ALH), Beat Frequency (BCF), Straightness (STR), and Linearity (LIN).

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